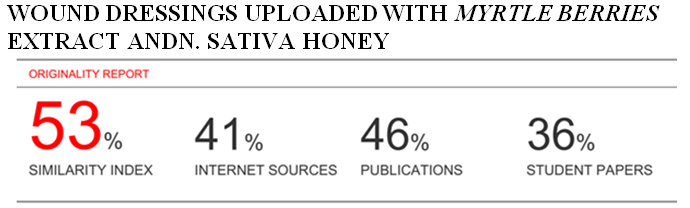
**Reviewer’s Comments**

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**WOUND DRESSINGS UPLOADED WITH MYRTLE BERRIES EXTRACT AND*N. SATIVA* HONEY**

**Abstract**

Wound dressings are frequently developed by introducing new products to target different aspects of the wound healing process. Many medicated dressings incorporated with natural extracts and chemicals have been developed. Chronic wounds could be invaded by many bacteria and*Pseudomonas aeruginosa*and *Staphylococcus aureus*are the most common. *S. aureus*and *P. aeruginosa* are usually detected in the higher layer of wounds or in the deepest region of wound bed, respectively.They can express many virulence factors affecting wound healing process andleading to severe infections and antibiotic resistance.Starch based impregnated gauze containing either *N. sativa* honey, myrtle berries hydro-alcoholic extract or a combination were prepared. There efficacy against both *P.aeruginosa* and *S.aureus*isolated from chronic wounds. *N. sativa* honey mixture was the most potent against *P. aeruginosa* with an inhibition zone diameter of 18.1±0.3 mm, while the myrtle berries hydro-alcoholic extract mixture was the most potent against *S. aureus*with an inhibition zone diameter of 18.4±0.5 mm. The prepared impregnated gauzes deliver a moist environment that helps wounds epithelialize more rapidly. In addition, honey and myrtle berries hydro-alcoholic extract provide antibacterial and anti-inflammatory properties that will accelerate the healing process of wounds.

**Keywords:** *Staphylococcus aureus*;*Pseudomonas aeruginosa*; *N. sativa* honey;myrtle berries hydro-alcoholic extract; starch based impregnated gauzes.

1. **INTRODUCTION**

A wound is defined as a simple or severe break in structure of an anatomical structure such as the skin and can outspread to other tissues [1]. Infection occurs in wounds due to competition with the host natural immune system and causes a delay in wound healing.The most common causes of infection are*Staphylococcus aureus*, *Pseudomonasaeruginosa*, *Streptococcus pyogenes*, and some *Proteus*, *Clostridium*, and *Coliform*species. The efficacy of topical solutions, creams or ointments for drug delivery to the wound is very low as theyrapidly lose their rheological characteristics due to the absorption of fluids[2].Traditionally,wound dressings are used to protect the wound from contamination [3], but they can be developed to deliver bioactive molecules such as to antimicrobial drugs wound sites.Wound dressings uploaded withnatural products, including the β-glucans, aloe, essential oils, honey, cocoa, and oak bark extracts are already being used in wound healing due to their antibacterial activity and wound healing properties [4].

Various parts of Myrtle [*Myrtuscommunis* L.] such as berries, fruitsand leaveshave been widely used as a traditional medicine for the treatment of several diseases due to their anti-inflammatory, antioxidant and antimicrobial properties [5, 6].Mnay components have been extracted from this herb and are considered to be the main biologically active componentsincluding polyphenols, myrtucommulone, semimyrtucommulone, α-pinene, 1,8-cineole, myrtenyl acetate, limonene, linalool and α-terpinolene[7]. High antibacterial activity ofethanol, methanol, and ethyl acetate berry myrtle extracts was observed when it tested against*S.aureus*, *P.aeruginosa* and *Escherichia coli* [8, 9].Some results have indicated that phenolic compounds and tannins greatly contributed to the antibacterial efficacy [10, 11]. In folk medicine, a decoction of leaves and fruits is used externally for wound healing [12].

Traditionally, honey has been considered to havetherapeutic properties since ancient times [13]. Results of different researches had previously proved the efficacy of honey against different types of microbes depending on many factors such as the type, natural structure of the nectar and the environmental conditions [14]. Bacterial resistance is less likely to develop as a result of treatment with honey because of the composition of honey which contains a number of different componentsresponsible for the antimicrobial efficacy [15]. This includes pH, sugar content, hydrogen peroxide levels and the presence of some phytochemicals, mainly phenolic compounds including phenolic acids and flavonoids [16].

Honey has also been proved to accelerate wound healing [17] by offering antibacterial activity, maintaining a moist wound environment that promotes healing, and providing a protective barrier to prevent infection [18]. Many researchers report that honey could be an effective dressing forthe treatment of different skin infections resulting from burns and wounds [19, 20].

In this study, the anti-bacterial effect of impregnated sterile gauzes containing myrtle berries extract and *Nigella sativa*honey was studied on both *P.aeruginosa* and *S.aureus*.

1. **MATERIALS AND METHOD**
   1. **Myrtle extract preparation**

Myrtle berries were collected from a mountainous region of Syria. 2 g of dried powders of myrtle berries were extracted by maceration in 100 ml of ethanol 50% for 2 hours [21].Finally, ethanol was evaporated using a rotary evaporator.

* 1. **Starch based gel preparation**

A starch based gel containing 20 g of starch, 20 ml of glycerol, and 100 ml of water was prepared first [22]. The solution was gently stirred until starch dissolved. It was then homogenized, heated for about 15 min at 80-85ºC andfinally cooled to room temperature.

Three different mixtures were prepared using10 ml of the starch based gel with 10 ml of *N. sativa*honey, 10 ml of myrtle extract or a combination of them at 1:1 ratio.

*N. sativa*honey was used in this study as it was found to be more potent on *P.aeruginosa*and*S.aureus* than other types of honey [23, 24].

* 1. **Impregnated gauze preparation**

Standard sterile gauze 3 inch by 3 inch was dipped intodifferent starch based mixturestill saturation and the excess solution was extruded by applying pressure. The hardening of the gel on the gauze was accomplished by refrigeration then the prepared impregnated gauzes were placed in sterile envelopes.

* 1. **Antibacterial efficacy**

*P.aeruginosa*and*S.aureus*were isolated from chronic wounds and tested for their antibiotic sensitivity as presented in **Tables 1** and **2**. Antimicrobial activity test was carried out using agar diffusion method on Muller Hinton Agar plates [25]. Bacterial isolates were spread on plates, and then a hole was punchedinto plates with a diameter of 6 mm. One hundred microliter of each mixture was introduced into the hole and the plates were incubated for 24 h at 37° C.The average of three cross sectional points of inhibition zone diameterwas taken as the inhibition zone.

**Table 1:Antibiotic sensitivities of *P. aeruginosa* isolate.**

|  |  |  |
| --- | --- | --- |
| **Result** | **Inhibition zone diameter (mm)** | **Antibiotic name** |
| Sensitive | 29 | Levofloxacin |
| Sensitive | 26 | Cefipime |
| Sensitive | 20 | Ceftazidime |
| Sensitive | 20 | Imipenem |
| Intermediate | 15 | Gentamycin |
| Intermediate | 15 | Doxycycline |
| Resistant | 10 | Ceftriaxone |
| Resistant | No inhibition zone | Amoxicillin+ clavulanic acid |

**Table 2: Antibiotic sensitivities of *S. aureus* isolate.**

|  |  |  |
| --- | --- | --- |
| **Result** | **Inhibition zone diameter (mm)** | **Antibiotic name** |
| Sensitive | 31 | Imipenem |
| Sensitive | 30 | Levofloxacin |
| Sensitive | 23 | Erythromycin |
| Sensitive | 22 | Meropenem |
| Sensitive | 20 | Tetracycline |
| Intermediate | 19 | Chloramphenicol |
| Resistant | 13 | Cefotaxime |
| Resistant | 11 | Linezolid |
| Resistant | 10 | Cefazolin |
| Resistant | No inhibition zone | Cefaclor |
| Resistant | No inhibition zone | Ceftriaxone |
| Resistant | No inhibition zone | Cefdinir |

1. **RESULTS AND DISCUSSION**

Application of conventional antibiotics is becoming more difficult due to several problems especially antimicrobial resistance and side effects. This has reinforced the use ofnatural alternative agentsto replace synthetic antimicrobials [26]. Accordingly, extensive research has been carriedout in order to assess the antimicrobial activity of the natural extracts and different types of honey which showed the ability to inhibit the growth of various pathogenic microorganisms [27].

**Table 3** shows the results of inhibition zone diameter of different prepared starch based mixtures on under-study microorganisms. Accordingly, the*N. sativa*honey mixture was the most potent against *P. aeruginosa* with an inhibition zone diameter of 18.1±0.3 mm similar to that of imipenem and ceftazidime, while the myrtle berrieshydro-alcoholic extract mixture was the most potent against *S. aureus*with an inhibition zone diameter of 18.4±0.5 mm similar to that of tetracycline and chloramphenicol.

**Table 3**: Sensitivity of *P. aeruginosa* and *S. aureus* isolates against different mixtures.

|  |  |  |
| --- | --- | --- |
| **Mixture** | **Inhibition zone diameter (mm)** | |
| ***P.aeruginosa*** | ***S.aureus*** |
| Honey | 18.1±0.3 | 11.2±0.3 |
| Myrtle extract | 15.3±0.2 | 18.4±0.5 |
| Myrtle extract with honey 1:1 | 13.6±0.4 | 15.6±0.2 |

The positive and potent effect of myrtle extract on *S. aureus* in this study is consistent with the results obtained by Taheri*et al.*[28] who had previously found that the concentration of 80 mg/ml of myrtle hydro-alcoholic extract showed thegreatest effect on the *S. aureus*bacterium with an inhibition zone diameter of 20.4±0.3 mm. Same results were obtained by Salvagnini who studied the effect of the oil and ethanolicextactof myrtle on different strains and reported that the ethanolic extract of myrtle has a positive effect on *S. aureus* with 12 mm inhibition zone [29]. GhlamhsynyanNajjar*et al.*acknowledged that the activity of myrtle extract on *S. aureus*strain is partly due to the stimulation of free radicals [30].

The efficacy of honey against different types of microbes has been previously proved in different researches [23, 24, 31]and bacterial resistance is less likely to develop as a result of the composition of honey which contains a number of different components [15].Results of different researchers proved that honey was more potent against *P. aeruginosa* than *S. aureus* which is consistent with our results.Boateng and NsoDiunase found that the zone of inhibition values for *P. aeruginosa* ranged from 26.3±0.6 mm for Manuka honey to 34±2.0 mm for Cameroon standard honey, whilst the zones of inhibition against S. aureus was not more than 18.7 ± 1.2 mm for Manuka honey [32].

As shown in Table 3, the combination between *N. sativa* honey and myrtle berries extract was effective against both *P. aeruginosa* and*S. aureus* with a diameter zone of inhibition of 13.06±0.4 mm and 15.6±0.2 mm, respectively.

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**Figure 1:** Impregnated gauze.

a. Impregnated gauze with 10 ml *N. sativa*honey. b. Impregnated gauze with 10 ml myrtle berrieshydro-alcoholic extract. c. Impregnated gauze with 10 ml *N. sativa*honey and myrtle berrieshydro-alcoholic extract mixture (1:1).

It is important to care properlyfor wound, whether it is a minor cut or a major incision. Dressings are a part of this process and are designed to be in contact with the wound, help in faster re-epithelialization, collagen synthesis andpromote angiogenesis [33]. Bioactive wound dressings incorporated with antimicrobials are one of the most important modern wound dressings developed to play an important role in healing process compared with traditional wound dressings used only for covering the wound [34]. Commercially available antimicrobial dressings includehoney-impregnated dressings, iodine-impregnated dressings, silver-impregnated dressings andchlorhexidine gauze dressing [35].

Misirlioglu*et al.* used honey-impregnated gauze for the treatment of a split-thickness skin graft donor site. The gauze showed alower sense of pain andfaster epithelialization time than paraffin gauzes and saline-soaked gauzes [36]. In the UK, dressings impregnated with Manuka honey were successfully used in the wound care clinic [37].

Subrahmanyam has shown in a randomized clinical study that residual scars decrease in patients treated with honey-impregnated gauze compared with those treated with amniotic membrane [38]. It was also proved that wounds dressed with honey-impregnated gauze showed earlier healing compared with silver sulfadiazene dressing in burn patients [39].

As presented in **Figure 1**, the prepared impregnated gauzescontain either *N. sativa* honey, myrtle berries hydro-alcoholic extract or a combination. The gauze can be cut to fit around the wound due to their soft elastic propertieswhich provides easy application and removal without any damage. They also deliver active compounds with anti-inflammatory and antimicrobial properties; and play an active role in the wound healing process. Starch based mixtures provide a moist environment in addition to a soothing and cooling effect.

**Conclusion**

Simple woven gauze although commonly used wo, they are known to be painful to remove, destructive to newly formed granulation tissue and provoke infection by leaving some fibers behind in the wound bed. A wide range of more appropriate dressings ensuring appropriate healing process has been available for a number of years such as medicated dressings. Plant extracts with antimicrobial and healing properties in addition to natural antimicrobial agents that were known to ancient cultures such as silver, honey and iodine are used for the preparation of medicated dressings. Although the perfect dressing is yet to be developed, wound dressings have evolved and further researches are still to be done.

**Conflict of interest**

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